ENZYMATIC STUDIES OF ADRENAL TISSUE

BY

ELDON DENZEL NIELSON

A.B., University of Utah, 1946

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
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ACKNOWLEDGMENT

The author wishes to express his sincere appreciation to Professor Carl S. Vestling for his interest and guidance throughout the course of these investigations.

He is also indebted to the Upjohn Company for the research assistantship held by him during 1947-1948.

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Introduction

The adrenal glands have been very extensively investigated in efforts to elucidate their role in essential life processes. Most of this investigation has been directed to the goal of determining the physiological factors governing their internal secretions and the effects produced by these secretions. *In vivo* studies have been numerous, as have chemical studies of the extraction products of these glands. A survey of the literature shows that relatively few enzymatic studies of adrenal tissue have been made.

The present study was undertaken in order to lay the foundation for an investigation of the enzymes of adrenal tissue. The eventual goal is to throw light on the enzymatic reactions involving the steroid hormones of the adrenal cortex. A study of certain aspects of the carbohydrate metabolism of the adrenals was considered to be a prerequisite to further work with this tissue.

Theoretical

The utilization of carbohydrate by animals is generally considered to proceed in two major phases: 1) the anaerobic phase (the Myerhof-Embden scheme), and 2) the aerobic-oxidative phase (the Krebs tricarboxylic acid cycle). The details of these metabolic pathways are given in the review literature (1) (2). A third scheme for the breakdown of carbohydrate is also known. The first step of this pathway, the oxidation of glucose-6-phosphate to phosphogluconic acid, was first studied by Warburg and Christian (3) with horse erythrocytes. The other steps of the so-called "hexose monophosphate shunt" were investigated in yeast extracts by Dickens (4) and by Warburg and Christian (5). Some probable intermediates after the initial oxidation to phosphogluconic acid are α-ketophosphogluconic acid and xylose-5-

phosphate. This process of oxidation followed by decarboxylation presumably leads to pyruvic acid as its final product.

Barker, Shorr and Malam (6) obtained indirect evidence of an alternate pathway of carbohydrate breakdown in animal tissue preparations through the use of iodoacetate to block the oxidative step of the Meyerhof-Embden cycle.

Experimental

All of the work reported here was done using the constant-volume Warburg apparatus to measure oxygen consumption. Determinations of glucose disappearance, pyruvate formation, and lactate formation were made to supplement oxygen consumption data.

Results And Discussion

In a system employing a ten per cent adrenal homogenate and added cofactors, cytochrome *c*, adenosine triphosphate (ATP) and diphosphopyridine nucleotide (DPN), the addition of any one of the following substrates caused a consumption of oxygen over that of the blank: glucose, fructose, mono- and diphosphorylated hexoses, succinic acid, α-ketoglutaric acid and fumaric acid. Pyruvic acid was not utilized as a substrate in this system.

The activity of the cortex and medulla were compared in this system. Such a comparison in the guinea pig showed that the Qo_2 of the medulla exceeded that of the cortex. In the hog, however, a qualitative difference was found. The cortex responded in a normal fashion, causing glucose to disappear and oxygen to be consumed. The medullary tissue caused a disappearance of glucose, but did not oxidize it. Autoxidation of epinephrine seemed to occur in preference to the oxidation of added substrate.

No differences could be detected when scorbutic guinea pig cortex was compared with normal guinea pig cortex in the system used.

When hexose diphosphate (HDP) was the substrate, two moles of pyruvate formed in the system for each mole of oxygen consumed. This is expressed as a ratio of moles of pyruvate formed to moles of oxygen consumed, or, more briefly, P:O ratio, which in this case is two. When glucose was the substrate, only small amounts of pyruvate formed, giving a P:O ratio of 0.1 to 0.5. Iodoacetate inhibited oxygen consumption and pyruvate formation by 92 per cent when HDP was the substrate. The inhibition of oxygen consumption by this poison was only 28 per cent when glucose was the substrate. Pyruvate formation was abolished under these circumstances. When fructose, glucose-1-phosphate, glucose-6-phosphate or fructose-6-phosphate were used as substrates, the results were similar to those obtained from glucose.

It was concluded that glucose was utilized by a metabolic pathway in which HDP is not an inetrmediate, and that in the unpoisoned system, 75 to 90 per cent of the oxygen consumption was due to reactions in this alternate pathway.

Consideration of the literature led to the conclusion that the alternate pathway of carbohydrate utilization present in the system employed is the hexose monophosphate shunt. It is not known whether the results obtained here are peculiar to the adrenal tissue or to the system employed.

Summary

1. A ten per cent adrenal homogenate in a system fortified with cytochrome *c*, ATP and DPN has been shown to oxidize glucose, mono- and diphosphorylated hexoses, succinate, α-ketoglutarate and fumarate.

2. Pyruvate was not utilized in this system.

3. Comparisons have been made of the enzymatic activity of cortex and medulla for both the hog and guinea pig.

4. Comparisons have been made of the enzymatic activity of the adrenal cortex of normal and scorbutic guinea pigs with this system.

5. In the system employed, glucose has been shown to be oxidized via a pathway in which HDP is not an intermediate. This pathway is presumed to be the hexose monophosphate shunt.

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VITA

The author was born December 4, 1920, in Hunter, Utah. He attended the public schools of the Granite School District and was graduated from Cyprus High School in May, 1940. He attended the University of Utah from the fall of 1940 until the spring of 1942. Following two years of employment, he reentered the University of Utah. He received the degree of Bachelor of Arts in Chemistry from the above institution in June, 1946. In March, 1946, he entered the Graduate College of the University of Illinois, where he held a teaching assistantship until June, 1947. He held an Upjohn Company research assistantship until September, 1948.

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